

## OVERCOMING CHALLENGES IN THE PRODUCTION OF HEPATITIS C VIRUS LIKE PARTICLES

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Hepatitis C virus (HCV) is a highly adapted human pathogen infecting nearly 3% of world's population. Notwithstanding the high efficiency of recently approved direct acting antivirals, important limitations in the administration and action of DAAs justify the development of a vaccine for HCV. Virus-like particles (VLPs) are a particular subset of subunit vaccines which are currently explored as safer alternatives to live attenuated or inactivated vaccines. In this work we explore the development of chimeric HCV-retroVLPs and native HCV-LPs as platforms for HCV antigen presentation.

Here we established a chimeric HCV-retroVLPs continuous production system based in recombinase mediated cassette exchange (RMCE) technology. We show functionality of HCV antigens displayed in retroVLPs and increased infectivity of HCV-retroVLPs produced with human serum supplementation [1]. Moreover the impact of host proteins present in retroVLPs was evaluated. Our results show that (i) tetraspanins are the major immunogens present in retroVLPs, (ii) that CD81 is highly incorporated in retroVLPs produced in HEK293 cells inducing specific B- and T-cell immune response in mice and (iii) that there is an increase in the diversity of tetraspanins in retroVLPs after CD81 depletion [2].

Important limitations are associated to the production of native Hepatitis C virus like particles (HCV-LP), namely the intracellular retention of assembled particles. In this work we demonstrated the importance of nonstructural proteins to increase HCV particles release and infectivity. We also explore the role of human serum as a releasing factor, increasing the number of secreted HCV-LP. A non-targeted proteomic analysis of secreted HCV-LP revealed the presence of HCV non-structural proteins as well as apolipoprotein-E, known to be present in patient derived HCV lipoviral particles, indicating a correct processing of secreted HCV-LP.

Overall this work further advances HCV vaccine development providing important insights on VLP cellular processing and impacting both upstream and downstream process development. The demonstrated enhancement of HCV-retroVLPs infectivity and the secretion of HCV-LP to the culture medium not only increases upstream yields, but also reduces significantly the amount of cellular contaminants derived from intracellular harvesting contributing to decrease the number of operation units at downstream.

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References: [1] Soares, H. R., et al. (2017) Biotechnology and Bioengineering (submitted) [2] Soares, H. R., et al. (2016) Vaccine. 34(13): p.1634-41